

## Flexible, Flat-form, Microfabricated Sensors for Substrate Concentrations and Enzyme Activities

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Microsensors (of micron dimension) are natural products of thin-film technology. Flat-form, flexible potentiometric membrane-based sensors were made quite early, circa 1969 in the Ektachem Project at Eastman Kodak Research Labs. Subsequently thin-film technology was applied in a few places where chemists were 'tolerated' by the predominant physicist population. Use of thin, flexible polyurethane insulator substrates were pioneered in our Laboratory beginning in 1989-1990. Multiple, flexible sensors of both amperometric and potentiometric types have been developed on 'polyurethane wafers' that require minimal calibration. In some biomedical applications using perfusion, electrodes can be calibrated in situ. Microsensors and arrays using thick-film fabrication on alumina bases have also been made successfully for flow-through blood analyzers. However thick-film technology is not suitable for most of our projects requiring electrode implantation into living tissue.

The design of stacked components of microsensors was soon found to require extensive synthetic chemistry and acquisition of especially hydrophobic forms of selective compounds. Characterization of new polyelectrolytes (functionalized polymers) was required, as well as physical testing: peel-tests, hydration tests, drift and shelf life tests. Biocompatibility tests occupied many years. The basic membrane electrochemistry required many special compounds to provide 'chemical recognition': hydrophobic ionophores, special plasticizers, polymeric coatings with controlled porosity and biocompatibility. Characterization required AC impedance and DC current-voltage studies, as well as calibrations. Theory and models from immiscible liquid-liquid electrolyte studies supported earlier models of potential-generation at sensor-sample interfaces. Similar tests were used for amperometric sensors that incorporated enzymes. The final set of devices was microreactors containing substrates for measurement of microinjected samples of enzymes. These were amperometric sensors turned inside out and used in the 'kinetic' mode.

Examples of flexible potentiometric microdevices are used to measure acidity in swine hearts during induced ischemia (lack of oxygen); simultaneous potassium and proton activities, together with lactate concentration in rabbit heart muscle during a total ischemia, and upon recovery to normal during perfusion with aerated electrolyte. Amperometric lactate sensors demonstrated surprisingly reproducible current responses for oxidation of enzyme-generated hydrogen peroxide. Other amperometric sensors, e.g. glucose and urea also performed well in moving systems. Evidently the reproducible membrane barriers on the platinum anodes are insensitive to motion. Other examples are devices to detect bacterial vaginosis (BV) using pH and amperometric putrescine sensors. The newer devices for enzyme activities began with a sensor for putrescine oxidase and a more selective BV test using measurement

of PIP (proline iminopeptidase). The initial reactor-sensors for enzymes showed amperometric responses that were non-linear at long times. Although this behavior is expected for some enzymes that are deactivated by generated peroxide, it was not expected for all enzyme-substrate pairs. The latest designs of kinetic cells by Lindner and Gyursanyi use a diffusion barrier separating the anode from the microkinetic chamber. In addition microfabricated micro-dot array sensors remove all traces of motion artifacts by enforcing hemispheric diffusion.

## References

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